

## Detection of *Trichomonas vaginalis* on Modified Columbia Agar in the Routine Laboratory

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**Broth culture of *Trichomonas vaginalis* is considered the “gold standard” for the diagnosis of trichomoniasis. Two studies were carried out to evaluate modified Columbia agar (MCA) for the isolation of *T. vaginalis* from clinical samples. Study I compared isolation on MCA to that on liquid medium with 889 vaginal samples. Out of 63 samples positive for *T. vaginalis* (7.1% of total), MCA identified 62 (98.4%) and broth identified 58 (92.1%). In study II, trichomoniasis was diagnosed within the scope of a screening program for a total of 39,585 men and women by culture on MCA and direct microscopy. Culture on MCA detected 199 (98.5%) and Gram staining detected 163 (80.7%) of 202 positive specimens. Wet-mount preparations used for symptomatic patients identified 103 (92.8%) of 111 cases. Culture of *T. vaginalis* from clinical samples on MCA is highly sensitive and reliable, as well as timesaving, and therefore suitable for screening of symptomatic and asymptomatic individuals.**

The parasitic protozoan *Trichomonas vaginalis* is a common pathogen that causes trichomoniasis and has been linked to preterm birth, acquisition of human immunodeficiency virus, infertility, and nongonococcal urethritis (4, 14, 16, 26). Males are most frequently considered to be asymptomatic carriers, which represent an important vector and reservoir (23). According to the population studied, the prevalence of infection in females varies from 5 to 50% in patients attending sexually transmitted disease (STD) clinics (4, 26). Although often observed in women with vaginal symptoms (26), trichomoniasis may be asymptomatic in up to 50% of infected individuals (8, 27). Hence, diagnosis has to be based on laboratory procedures (23), such as direct microscopy and culture. Most frequently, the saline wet-mount preparation is used for observation of motile organisms. Different staining techniques, including those using Gram stain, Giemsa stain, Papanicolaou (Pap) smear, and acridine orange (1, 26), and diverse molecularly based diagnostic methods, such as hybridization assay and PCR, have been employed to detect *T. vaginalis* but vary widely in their sensitivities and specificities (5, 15, 17–19, 21). Thus, broth culture is still considered the “gold standard” for diagnosis of trichomoniasis (1, 24). For this purpose, various liquid culture media have been developed (1, 2, 10, 24, 25). However, identification of positive samples requires frequent microscopic observations for up to 7 days.

To evaluate the suitability of a solid medium cultivation technique in the routine laboratory, two studies were carried out. In study I, modified Columbia agar (MCA) was compared to a commercially available liquid medium in its ability to support trichomonal growth from clinical specimens. Subsequently, in study II, culture on MCA and direct microscopy were used to screen a low-risk population for *T. vaginalis*.

Finally, the reliabilities of the methods employed were determined.

### MATERIALS AND METHODS

**Study I.** A total of 889 women attending the Outpatients' Center in Vienna, Austria, because of symptoms and/or signs of infections of the genital tract, or for contact tracing, were included in the first study period between 1994 and 1995. Four swab samples from the posterior vaginal fornix were collected from each woman and processed at the bedside. The first two swabs were used for wet-mount preparation and Gram staining, respectively. Trichomonas medium and finally MCA plates were inoculated with the third and fourth samples, respectively.

To investigate the ability of broth culture and MCA plates to grow different concentrations of *T. vaginalis*, an in vitro experiment was performed. Standardized inocula of  $10^0$ ,  $10^1$ ,  $10^2$ ,  $10^3$ , and  $10^4$  organisms per ml (final concentrations) were inoculated into 5 and 17 ml of trichomonas broth as well as onto MCA plates. For quality control, *T. vaginalis* ATCC 30001 was processed in parallel and with all lots of media produced.

**Study II.** Altogether, 39,585 individuals (13,195 men and 26,390 women) presenting at the STD center in the years between 1996 and 2000 for routine microbiological examinations and for suspected infections of the genital tract with or without symptoms were screened for *T. vaginalis*. Trichomoniasis was diagnosed by culture on MCA and by Gram staining of vaginal samples from women and urethral specimens from men. For symptomatic patients, immediate wet-mount preparations were used. Specimens were sampled and processed as described above.

**Cultivation and identification.** Trichomonas medium (Oxoid Ltd., Basingstoke, Hampshire, England) was dissolved, autoclaved, and mixed with 10% sterile, inactivated lamb serum (PAA Laboratories, Linz, Austria), 1% dextrose (Sigma Chemical Co., St. Louis, Mo.), 1% malt extract (Oxoid Ltd.), and 1 ampoule of chloramphenicol selective supplement (Oxoid Ltd.). A 17-ml volume was dispensed into tubes with screw caps to ensure anaerobic growth conditions and to keep organisms viable until the end of incubation. MCA was prepared as follows: 13 g of Columbia agar (Oxoid Ltd.) was dissolved in 320 ml of distilled water, and the solution was adjusted to a pH of 6, cooked for 20 min, autoclaved at 121°C for 5 min, and cooled before the addition of 50 ml of sterile, inactivated lamb serum (PAA Laboratories) containing 4 g of dextrose (Sigma Chemical Co.), 4 g of malt extract (Oxoid Ltd.), 15 ml of Baneocin (250 U of bacitracin/ml and 5,000 U of neomycin/ml; Biochemie, Vienna, Austria), 10 ml of nystatin solution (10,000 U/ml; Biochrom KG, Berlin, Germany), 4 ml of penicillin-streptomycin solution (10,000 of penicillin/ml and 10,000 µg of streptomycin/ml; Gibco BRL, Life Technologies, Paisley, Scotland), and 1 ampoule of chloramphenicol selective supplement (Oxoid Ltd.). Approximately 10 ml of the resulting mixture was poured into 6-cm-diameter petri dishes. Both media were stored at 4°C for no longer than 1 week and allowed to reach room temperature before

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TABLE 1. Identification of *T. vaginalis* in a total of 39,585 individuals by Gram staining, culture on MCA, and wet-mount microscopy

Test result	No. of samples			Result of:		
	Total	Men (n = 13,195)	Women (n = 26,390)	Direct microscopy		Culture on MCA (n = 39,585)
				Wet mount (n = 111) <sup>a</sup>	Gram stain (n = 39,585)	
Positive	202	2	95	+	+	+
			3	+	—	+
			1	+	+	—
			2	+	—	—
			1	—	+	+
			6	—	—	+
			64	ND <sup>a</sup>	+	+
			4	ND	—	+
Negative	39,383	13,188	26,195	ND	—	—

<sup>a</sup> ND, not done in clinically asymptomatic patients.

use. Immediate incubation of inoculated broth and agar plates, which were held under anaerobic conditions (Anaerocult P; Merck KGaA, Darmstadt, Germany), was carried out at 37°C for 6 days.

Identification of *T. vaginalis* was achieved by observation of motile organisms in wet-mount preparations and by use of Gram-stained smears. Macroscopic evaluation of all cultures for turbidity in the case of trichomonal growth and of slide preparations from broth cultures was performed regularly. MCA was examined microscopically (magnification,  $\times 100$ ), and results were confirmed by using a saline wet mount. Colonies of *T. vaginalis* on MCA cause a change of pH resulting in a muddy appearance of the plates. The characteristic puzzle-like structures caused by trophozoites lying close to each other can be observed most easily at the edge of the colony.

## RESULTS

**Study I.** Out of the 889 women enrolled in study I, a total of 63 (7.1%) were positive for *T. vaginalis* by direct microscopy and/or culture. Growth of *T. vaginalis* was observed in 58 (92.1%) of 63 samples in broth culture and in 62 (98.4%) of 63 specimens in culture on MCA, resulting in an overall accordance of 93.5%. Altogether, 10 (17.2%) of 58 positive cultures in trichomonas broth showed low numbers of organisms, whereas 2 (3.2%) of 62 positive MCA plates showed low numbers of organisms. One positive sample was detected by direct microscopy only, while this method missed a total of six infected samples that were identified by culture on MCA. With broth culture, three of these samples were positive and another two showed low numbers of organisms, leaving the infection in one specimen not detected. A total of five infections would have been missed if broth culture only had been performed. No sample positive on liquid medium but negative on MCA plates was observed.

**In vitro experiment.** MCA plates were positive with all standardized inocula within 6 days of incubation. After 24 h, plates that had been inoculated with  $10^2$  trichomonads per ml or more were positive, while inocula of  $10^1$  trichomonads gave positive results after 72 h. All broth cultures inoculated with  $10^0$ ,  $10^1$ , or  $10^2$  trichomonads per ml stayed negative for the whole examination period, regardless of the volume used. Both the 5-ml volume and the 17-ml volume showed positive results with inocula of  $10^4$  and  $10^3$  trichomonads after 24 and 48 h, respectively.

**Study II.** Screening for *T. vaginalis* performed with a total of 39,585 men and women attending the STD center identified

202 infected individuals (7 men [0.05%] and 195 women [0.74%]), revealing a prevalence of 0.5%. Table 1 shows the results obtained for all specimens collected. Gram staining of smears and culture on MCA were performed to detect trichomonads in both asymptomatic and symptomatic patients. Wet-mount preparations were used for samples from 111 symptomatic patients (3 men and 108 women), or 55% of infected individuals.

Culture on MCA detected 199 (98.5%) of 202 infections in symptomatic as well as asymptomatic individuals, with sensitivities of 100 and 97.3%, respectively (Table 2). Infections in three samples could be detected by direct microscopy only, that in one specimen was detected with a wet-mount preparation as well as Gram staining and those in two samples were detected by using wet mount exclusively. Gram staining identified 163 (80.7%) of 202 infected persons, with infections in 12 symptomatic and 27 asymptomatic individuals not detected. For 111 samples from patients with clinical symptoms, wet-mount preparations detected infections in 103 (92.8%) specimens but failed to detect 8 positive specimens, and only 1 of these samples was correctly diagnosed by Gram staining. For 91 samples from *T. vaginalis*-positive patients, wet-mount preparations were not used due to the lack of clinical symptoms. Performance of wet-mount microscopy in addition to culture increased the detection rate from 97.3 to 100%.

TABLE 2. Sensitivities of different diagnostic techniques in symptomatic and asymptomatic individuals with trichomoniasis

Infected patients	% Sensitivity <sup>a</sup>		
	Wet mount	Gram stain	MCA
Asymptomatic (n = 91)	ND	70.3 (64/91)	100 (91/91)
Symptomatic (n = 111)	92.8 (103/111)	89.2 (99/111)	97.3 (108/111)
Total (n = 202)	ND	80.7 (163/202)	98.5 (199/202)

<sup>a</sup> Values in parentheses are the number of positive patients relative to the total number of patients tested. ND, not done in clinically asymptomatic patients.

## DISCUSSION

A low rate of infection with trichomonads, only 0.5%, was detected in this study population, and this rate is in accordance with observations in other countries (6, 26). However, *T. vaginalis* should be included in STD screenings, not only because it is associated with gonorrhea and *Chlamydia trachomatis* infection (20, 27), but also because screening for *T. vaginalis* is considered an achievable strategy to reduce the incidence of human immunodeficiency virus (9). The requirements of diagnostic techniques for large-scale screenings with respect to sensitivity, specificity, and duration of test results are very high. Although enzyme immunoassays, PCR, and hybridization-based detection of *T. vaginalis* meet some of these requirements, performance of these tests is restricted to specialized laboratories because of the high costs involved. Moreover, these tests may not always be commercially available. Although wet-mount microscopy is a cost-saving method, a sensitivity of approximately 40 to 80% reduces its reliability as a diagnostic tool (15, 21, 26, 27). The data presented in this study reveal a sensitivity of 92.8% for wet-mount microscopy performed on samples from symptomatic patients. However, the reliability of clinical symptoms is low, because in the present study as many as 91 individuals, or 45% of infected patients did not show typical signs of infection. An unfortunate limitation of this study due to the great expense of a large-scale screening is that there are no data available on wet mount results for asymptomatic patients. Most likely, these individuals harbor small numbers of trichomonads and/or a reduction in, or loss of, the parasites' motility causes a negative outcome with this test (15, 21, 23, 26, 27).

Pap smears are often performed in gynecologic screenings and have a reported sensitivity of approximately 60 to 70% (26, 27). However, an error rate of about 48% due to false-negative and false-positive results has been observed when Pap smears are used as the only diagnostic method (22). Staining techniques, such as with acridine orange or Giemsa stain, show sensitivities of about 60 and 50%, respectively, while Gram staining is not recommended to diagnose trichomoniasis because organisms may appear similar to polymorphonuclear leukocytes (26). However, Gram-stained smears are frequently implemented for microbiological examination in the routine laboratory. In this study, results obtained with Gram staining demonstrated sensitivities of 89.2% in the symptomatic patient and 70.3% in the asymptomatic patient. So, in contrast to published data, Gram staining was equivalent to the wet-mount or Pap smear technique (26) and may as well be substituted for other diagnostic techniques if immediate microscopic examination is impracticable. On the other hand, additional Gram staining did not significantly increase the detection of *T. vaginalis* by culture. Finally, the outcome of microscopic examinations of stained smears is subjective and dependent on the experience of microscopists, and results need to be confirmed by culture.

The reported sensitivity of culture in liquid media, such as Diamonds and Trichosel, is 85 to 95% (21, 26). The most important disadvantage of broth culture is the indispensable and time-consuming slide preparation needed for identification of positive cultures over a period of up to 7 days. Culture systems, such as the InPouch TV test (1), the plastic envelope

method (3), or the microtiter tray culture described by Hill (11), have been tried to facilitate examination. Semisolid or so-called pour-plate culture techniques to isolate and/or quantitate *T. vaginalis* organisms have been described previously (7, 12, 13, 24). However, these techniques are not suitable if large numbers of clinical specimens are to be examined. MCA has been developed to overcome these problems and is to our knowledge the first solid medium suitable for the isolation of *T. vaginalis* from clinical samples in the routine laboratory. Data presented in this study reveal that MCA has a sensitivity of 98.5% in detection of *T. vaginalis* in a low-risk population comprising symptomatic as well as asymptomatic patients. The in vitro experiment demonstrated MCA to be superior to broth culture in detecting low numbers of trichomonads, confirming the results of this study. Compared to broth culture, the agar plate technique reduces the risk of missing positive specimens by making possible the microscopic examination of the whole clinical material obtained. This solid medium is a timesaving culture technique because it eliminates the need for slide preparations, and it is favorable for screening a low-risk population if more than one sample is inoculated onto a single plate. Since wet-mount and stained-smear methods show low sensitivities for detection in asymptomatic individuals, culture of *T. vaginalis* can be recommended as the only diagnostic method for this patient group and should therefore not be replaced by these tests. However, this culture technique has a turnaround time of 2 to 6 days. Sometimes rapid tests, such as enzyme immunoassays or PCR, are preferred. An evaluation of the methods and MCA will have to be carried out in the future.

In summary, MCA performed as a highly sensitive, reliable, and easy-to-handle culture technique in the routine laboratory for diagnosis of *T. vaginalis* infections in clinical samples from symptomatic as well as asymptomatic individuals and may provide a suitable screening method for trichomoniasis.

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